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Impact of Transgenic Fishes on Aquatic Ecosystem and Biodiversity of Freshwater Teleost

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Abstract*: Critics showed objections on the genetic engineering process on the some points whish were both ethical and ecological. This happened because it was sure that these genetic engineering processes must have been causing the gene flow and raise alarm against intellectual property rights. Problems also evolved on using GMOs in the form of food because fishes are being used as a nutritive food worldwide. So it raised a question in the mind of critics that it can cause many forms of lethal allergies to the consumers as the GM fishes were labelled. These issues caused a check over the trade of these GM fishes internationally. Thus due to all these prominent issues FDA has a power to restrict these GM fishes as 1) food 2) conditions in which they are used 3) a check after the approval. Public is confident if the above written three points have positive outcome. So these three points are the backbone for the approval of GM as food for public. So the potential research benefits of genetically modified fish will be fruitful only if these transgenic fish are kept separate from genetic pool of the wild species. There is possibility of transmission of the transgenes to the wild fishes which can make these transgenes a permanent resident of an environmental ecosystem.*

Keywords: Transgenic, GMO, Germline

Introduction

Many plants and animals have been genetically modified such as frogs, fruit flies, see urchins laboratory mice and farm mammals such as cows, sheep and pigs. In plants introduction of transgene have been performed by infecting the plants with *Agrobacterium tumefaciens* and also by other physical means physical means. Injection of foreign DNA in the animals is mainly done by injecting the foreign DNA into the pronuclei of the fertilized eggs. Now these pronuclei are either matured by incubating it in *in vitro* or by pseudo pregnant females. This introduction of the foreign gene into the animal host is random i.e. the foreign gene will incorporate itself at any position in its genome. These foreign DNA also require some promoters which is very much essential for their expression. The transgenic animals play an important role in applied biotechnology. These transfected animals are very much helpful for studying early vertebrate development and also its molecular genetics, functions of hormones and the oncogene study can also be performed. Background of breed stock for animal husbandry and also for fish aquaculture Since 1985 many experiments have been performed to transfect the fishes. Techniques such as electroporation and microinjection have been used for introducing the foreign DNA into the fertilized eggs. In case of genetically modifying the fishes following steps are taken:- a) selection of the particular fish species b) the construct of gene which is taken should be specific b) last but the most important step is that the transgene is introduced at an embryonic stage transgenic of the selected fish species.

Selection of Fish Species

Many genes transfer experiments have been conducted on different species of fishes, which includes Rainbow trout, Goldfish, Comman carp, Salmon and many more (Aliye, 2003). On this gen transfer studies the embryos of some fishes proved to be better for gene transfer studies than the other fish species.

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Japanese Madaka (*Oryzias latipes*) and Zebra fish (*Barchydania rerio*) have a life cycle of 3 months starting hatching to the mature adults. These fishes also produce hundreds of eggs on a regular basis. They have large eggs and have a thin chorion that is semi transparent. This feature is very much favourable for transfecting the eggs. They also have a disadvantage of a having a small body size, due to which they are undesirable for some biochemical and endocrinological analysis. Channel fish, Rainbow trout and Salmon have large body size, which suits to be a better model fish for studying transgenesis. But they have longer duration of maturation and also one spawning cycle per year, restricts the progress of research in the field of genetic engineering.

For Introduction of Foreign Genes in the Fishes There are Numerous Methods

1) Selection 2) Gynogenesis 3) Androgenesis 4) In breeding 5) Sex reversal and breeding 6) Breeding and Nuclear Transplantation 7) Cross breeding which are intraspecific 8) Hybridization which is Intersepcific 9) Polyploidy 10) Transgenesis and Nuclear Transplantation.

Genetically Transforming a Zebra Fish

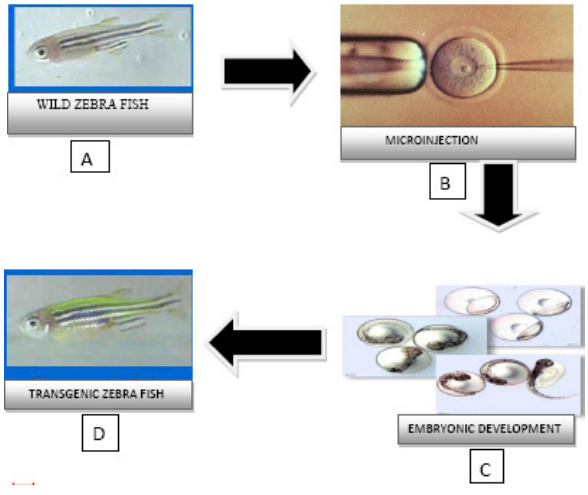

Fig. 1 A) Design a transgenic gene construct. **B)** Introduction of DNA into the eggs.**C)** Screening for transgenic fishes. **D)** Breeding to establish stable transgenic lines.

Species that have been Worked Upon

Numerous fishes have been genetically engineered which includes loach, which is a non-commercial, also *Misgurnus anguillicaudtis* (Agnes *et al., 2002*), Zebra fish, topminnows (Bartley *et al., 1986*). Trangenic rainbow Zebra fish is also being developed for the ornamental fish industry (Gang wu *et al.*, 2003). Experiments have been performed. several non-commercial model species are also been targeted for performing transgenetic experiments such as the medaka, *Oryzias latipes* (Bartley *et al.*, 1986), loach, *Misgurnus anguillicaudtis* (Agnes *et al.*, 2002), top minnows and zebra fish, although Gang Wu *et al*., (2003) have developed transgenic rainbow zebra fish for the industry of ornamental fishes. Experiments upon farmed fish species including goldfish (Chatakondi *et al*., 1995), common carp, rainbow trout (Chourrout *et al.*, 1986) silver carp, mud loach, are also been performed.

Traits determining Net Fitness

- ✓ Chances of surviving to sexual maturity.
- ✓ Chances of surviving to procreate.
- ✓ Number of eggs successfully fertilized by male sperms.
- ✓ Number of eggs produced by a female sperm.
- ✓ Success at securing mates.
- ✓ Age at sexual maturity.

Review of Literature

Genetic transfer with fish began in the mid 1980's was the year when the gene transfer in the fishes started using the technique called as Microinjection (Zhu *et al.*, 1985, Chourrout *et al*., 1986, (Dunham *et al*., 1987). Zhu *et al.*, (1985) firstly introduced a foreign gene through microinjection and publishe his report of "Transgenes Microinjected into the Fertilized Eggs of Goldfish". In every transgene experiments the foreign gene is injected into the cytoplasm of one to four celled embryo by the technique-microinjection. It's a tedious to

view a pronuclei of the oocytes which have to be extracted 9 hours before ovulation. In almost all fish gene transfer experiments, the foreign gene was introduced into the cytoplasm of one-to- four cell embryos by microinjection, as pronuclei which are very difficult to visualize in live one-cell injected the oocytes of Medaka (*Oryzias latipes*), which had been removed from the ovaries nine hours before ovulation (Gang *et al*., 2003). The efficiency of microinjection is 5% only so it is a slow and tough procedure which needs a skilled hand. An unskilled person handling this technique can lead to egg mortality. With microinjection many other procedures were used parallel such as retroviral integration and electroporation, micro projectile bombardment, sperm mediated transfer etc., resulted to be an accurate techniques. Electroporation uses a short electric current or impulse which temporarily makes the plasma membrane of the target cell porous so that the foreign gene can get introduced into the cytoplasm. It has a buffering solution which have the foreign DNA (Du *et al*., 1992). He demonstrated the efficiency of this procedure is 30-100% more efficient compared to microinjection. Walker (1993) also found the same results that the rate of gene incorporation is high in electroporation as compared to microinjection in Channel catfish embryo. Gene integration and hatching percentage are the two factors upon which the efficiency of the gene transfer depends (Mc Andrew and Napier, 2011).

Due to this the electroporation technique is a potent method for mass production of transgenic fish. Another procedure was used in which a Retroviral vectors are being used which is having a viral envelope of vesicular stomatitis virus (Burns *et al*., 1994), and used to genetically modify fish (CuiZhang *et al*., 2007). But sometimes these vectors resulted in gene silencing (Sarmasik *et al*., 2001). He performed gene transfer experiments in crayfish and topminnows, *Poeciliposis lucida*. Another vector known as pantropic retroviral vector which was derived from the vesicular stomatitis virus and hepatitis B virus which was used to infect the mammals and insects. This vector adheres to the plasma membrane of the host. A solution of the vectors which was prepared one month before was used to inject in the immature gonads of crayfish *(Cambarus)* and topminnows (*Phoxinus phoxinus* .After that these transgenic individuals were allowed to breed with the normal ones which gave 50% transgenic offsprings. This procedure gave a good results but due to the viral construct introduction into the fish was not gullible for the general public. Replacing the retrovirus the transposases were used for the safety reasons but it did not proved to be a better option for live-bearers. Gene knockout experiments were also used to replace original gene with a mutant gene. Ribozyme technology was also used which inactivates the expression of gene using the antisense approach. One more technique was used for silencing a gene post-transcriptionaly using RNA antisense construct (Rahman MA *et al*., 1998). Expression of GFP was silenced using antisense RNA and double stranded DNA in transgenic zebra fish *(Danio rerio)* (Wei *et al*., 1992*)*. The transgene which is used to genetically modify a species initially replicates in the cytoplasm of the developing embryo due to which it is used to get disappear as the growth continues (Houdebine and Chourrout 1991). Mosaicism occurs in the developing embryo as it is not possible to insert the foreign gene in a single celled embryo (Du, 1922), due to which all the tissues of the developing embryo will not contain the foreign gene construct. Whether or not the transgenes have been passed to the F2 generation of the progeny occurs at Mendelian frequencies (Ayoola *et al*., 2008).

Transgenic Aquatic Organisms: Food Safety

The uneducated people are generally unaware of biology and that how the food

which they consume are grown or processed, therefore it is very necessary to make these people understand about the advantage and disadvantages of the food which they consume (FAO 2001). Berkkowtz and krypsin Sorensen (1994) tried to explain the pros and cons related to consuming the transgenic fish. There is a risk in consuming the transgenic fishes because they will be producing the proteins which are obviously not natural or wild type proteins. Consuming it may cause allergies which can be lethal, also they can activate viral sequences. In all these risks allergenicity can prove fatal. The protein content of the soybean was increased by genetically modifying it with the help of a transgene from Brazil nut which proved to be allergic to some group of peoples (Chatakondi *et al*., 1995). The intake of Tilapia growth hormone did not shown any change on the health of animal (Frank, 1997). In an experiment 22 humans were allowed to feed on tilapia (transgenic hybrid *Oreochromis hornorum*) that expressed GH transgene (Thomas, 1990). The transgenic Tilapia grew faster compared to the control experiments with twice the rate of control fishes. Creatinine, glucose, cholesterol, leucocytes and erythrocytes were determined. No change in the total protein modifications of the blood protein was observed. So it was concluded that the consumption of transgenic Tilapia was non-bioactive in the primates. Intake of growth hormone stimulates increase in the weight of kidney and spleen and also it causes erythropoiesis or lymphopoiesis (Wei *et al*., 1992). It is also associated with growth changes, fluid retention and changes in blood volume (Thomas, 1990). These depicted changes were not observed on the humans. Transgenic salmon transfected with GH and SIGF genes also proved biologically inactive upon feeding (Dunham 2004). Also cooking the fishes as a food denatures any protein which might affect the human health. In the end it was inferred that consuming transgenic Salmon and Tilapia was not hazardous to humans.

Transgenic Fish in Research and Development

a) US and European scientists have genetically modified the Atlantic Salmon by increasing the growth rate and food conversion efficiency by insertion of Chinook Salmon growth hormone genes that is switched on year-round, thereby fostering year round, rather than mainly summer growth. The intended use of this product is human consumption. The status of this product is seeking regulatory approval. Its method has been patented. FDA is reviewing application for commercial use.

b) Canadian & US researchers have introduced Sockeye GH genes in Rainbow Trout. It has been used in human consumption. The status of this product is that it is being used as a model for other research.

c) Cuban research has genetically modified Tilapia genetically by inserting Tilapia growth hormone genes linked to a promoter derived from Human Cytolomega Virus (CMV). Intended use is human consumption. It is seeking regulatory approval.

d) China improved disease resistance in Carp by inserting a human interferon gene.

e) Chinese and Canadian research has increased cold tolerance in Gold fish by inserting ocean pout anti-freeze protein gene. Its intended use is in ornamental fishery, feed and research and research purposes.

f) UK and US research have produced clotting factor VII after insertion of human gene for clotting. Its intended use is in pharmaceutical products.

g) US research have increased the growth rate and have done non-specific bacterial immunity in channel Catfish Using Salmon Growth Hormone(GH) genes.

Some of the Transformed Fishes

Fig. 2 Fluorescent transgenic zebra fish as new type of ornamental fish. The left picture shows three red fluorescent *mylz2:RFP* transgenic fish (also known as Gold Fish TM), whilst in the right three green fluorescent mylz2:GFP transgenic zebra fish and two wild type zebra fish (labelled with WT)

Problems Faced in Applications

The process of genetically modifying fishes started since 19 years back, but the progress of this work stopped because of many reasons. Firstly being the lack of funds which hindered the ongoing research process. Due to this the facilities lacked and it confined the transfer of transgene into the fishes. Transfecting the fishes creates mosacism which lengthens and causes the process of research to slow down (Liu *et al*., 1990). It was seen that these transgenic fishes were relatively long generation time as compared to the wild ones. Also it is not confirmed that the transgene is confirmly get incorporated into it right loci. Epistatis is the outcome of this random incorporation of foreign gene. Scientists have to optimize the phenotype and the expression of the genes which proved to be a time taking process. So it needed patience and at the same time a good facilities. Also some of the transfecting experiments were not feasible because the embroyonic stem cells were not available which hindered the trial processes for gene knockout technologies. Some viral promoters have also been used which proved to be a good result giving process. In spite of these problems researchers have given good results in this field. The main problem is the acceptance of public and there should be almost nil affect of this technology on the environmental risks issue.

Ongoing Research Activities and Efforts Made

Nowadays genomics have been proved as the largest and important global activity in research and it is very much relevant to the future as well as current scenario of transgenic. A large amount of research is going on these days regarding the regulation of gene and its expression, gene mapping is being done after its isolation and sequencing. The gene transfer technique and application in fish currently exists in a much smaller amount now a days. But some majot projects are going upon the examination of growth enhancement, incorporation of disease resistant gene in the fishes, temperature tolerance etc. Monitoring the environmental risks is the main moto of the researchers nowadays, fitness measurement and determining the food safety. For a better transgenic expression a tissue specific expression proves to be the most promising technique which is thought to be a great performer in future. In Zebra fish several tissue specific promoters incorporation have given a fruitful results (Gong *et al*., 2002). It includes keratin 8 specific promoters of epidermis, a muscle specific myosin promoters were also incorporated. An elastase B which is specific for exocrine cell also proved an better option for transgenicity. An enzyme called HSP70 gene that is inducible too plays an important role in protein metabolism. The inducible HSP70 gene that encodes an enzyme playing an essential role in protein metabolism. It has been isolated from *O. mossambicus*. It increases the rate of transcription when the fish is being given a heat shock.

Applications of Transgenic Fishes

Right now not an excessive research is going on upon the transgenic fishes but investments are being made on genomic research and the agencies which are funding these projects because there is no controversy linked with. There are two important processes have been learned from this are and that are gene transfer and marker assisted selection. A powerful method of aquaculture had benefited the genomic part sequestering its problems. The main hindrance in the way of application of transgenic fishes is their commercialization. Fishes that will be resistant to a particular disease will be a beneficial addition to assist the environmental issues. So the disease resistant fishes will be profitable which will increase the production and efficiency. With the transgenic approach there will be an increase in the growth rate of fishes (Dunham *et al*., 2002) as it helps in aquaculture production. Through transgenic fishes the reproduction can also be controlled. If this proves better in the future then it will be a big achievement in the field of recombinant DNA technology.

Through this problems of genetic biodiversity and biodiversity will be solved in future, since it applies a safer application of genetic engineering. The most beautiful contribution of transgenic fishes is the change in the colour of ornamental and aquarium fishes. Recently, the first application of the transgenic fish production is the alteration of colour of aquarium and ornamental fishes having gene of fluorescent pigment. But there is a drawback of these genetically modified fishes that they will not be able to survive in the natural habitat so it will create a confinement and environmental issues.

Risks and Benefits on Exploitation of Transgenic Fish in Aquaculture

Risks	Probability
Transgenic fishes causes health issue-	Very low
Transgenic fishes do not compete with non-transgenic-	Low
Transgenic fishes on release or escape becomes undesirable-	Requires control
Transgenic fishes on breeding with wild individuals results in permanent presence of transgene	Requires control
Transgenic fish on escape die out quickly, affect ecological--	Low

Benefits	Probability
Transgenic fish results in low cost, faster growing strains required for aquaculture-	High
Transgenic fish provide disease resistant strains-	Low in short term
Transgenic fish have strains which are freeze, cold and salinity tolerance-	Fairly low in short-term

Biotechnological Approaches and Safety Evolution

- Biotechnology could be used to reduce the amount of mercury and other toxic compounds that fish accumulate from their environment.
- Things can be done to remove the allergens that make shellfish intolerable for some consumers.
- Transgenic fishes can be so designed that it can be used to detect environmental contaminants.

Impact of Transgenic Fishes on Environment

Many millions of farmed fish can escape from the environment in which they are under test every year and contaminate native populations. It is an inevitable phenomenon in which transgenic fish escape from the aquaculture or from the field of trial parameters. Transgenic fishes have greater effect on the natural environment than the hatchery-reared non-transgenic fish have fast growing transgenic fishes can bring a major change in commercial fish farming and can save the wild fish stock from overexploitation. On the other hand, what happens if those transgenic fishes find a way to get introduced in the natural environment and can hamper the ecology of that habitat? That will be a big threat for the natural population of fishes. Introduction of a transgene to a natural stock of fishes can compete with the natural species of that habitat for food and other resources. By furnishing a new gene to a fish from some other organisms, called as transgenes, researchers have succeeded in producing transgenic fishes that can grow comparatively fast compared to their wild forms. A new gene can also make a fish resistant to colder conditions. As the transgenic fish gives higher production and better yields, there are better chances for commercial fish farming. However, these transgenic fishes introduced to the natural environment can also entail risks on the natural environment. Fishes are made resistant to the toxins for their survival in the environment having toxins which are hampering the growth aspects of the aquatic life**.** Marketing of these fishes will cause ill health to the consuming group of population. The natural breeders are also under threat, if these transgenic fishes get established in the natural stocks, they will definitely try to compete with the natural breeds. Growth rate enhancements have also been done by transfecting the fish with growth hormone genes. Large body size fishes compete well with the small sized one, causing decrease in the population of small sized one. Faster growth rate in transgenic Atlantic Salmon would mean that they could be at an advantage both in inter-specific and intra-specific contests in rivers. The transgenic fishes entering the river through escapes from fresh water hatcheries, would impose a large effect on the river ecosystem, because there will be always some new fishes entering that ecosystem, some of them being very large compared with wild fishes. Transgenic escapees would be larger than the wild fish and would be more dominating on the spawning grounds.

Food Safety Issues

Commercializing transgenic fishes can undoubtedly deliver higher production and better yields. But it also entails risks effects which are undesirable on natural environment, such as genes inserted to promote disease resistance may cause transgenic fishes to intake toxic substance (like mercury) at a higher rate and this intake of toxic substances is transferred to the consumers. Majority of fishes are transfected with growth genes. So

there are the chances that the large doses of growth hormone may pose health risks if it is consumed in raw form or uncooked form like sushi. Approximately 90% of the food allergy cases are due to the consumption of eggs, fish, shellfish, milk, peanuts, soybeans, tree nuts and wheat. If proteins used in the production of transgenic species originate from one of these eight sources, they may be the potential reason for allergic reactions among the consumers. This is the reason researchers at the University of Gothenburg area are authorised by European Union (EU), to have a check on the risks of GMO within fish farming.

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